PRELIMINARY COMMUNICATION

Effect of Shock Wave–Facilitated Intracoronary Cell Therapy on LVEF in Patients With Chronic Heart Failure

The CELLWAVE Randomized Clinical Trial

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Importance The modest effects of clinical studies using intracoronary administration of autologous bone marrow–derived mononuclear cells (BMCs) in patients with chronic postinfarction heart failure may be attributed to impaired homing of BMCs to the target area. Extracorporeal shock wave treatment has been experimentally shown to increase homing factors in the target tissue, resulting in enhanced retention of applied BMCs.

Objective To test the hypothesis that targeted cardiac shock wave pretreatment with subsequent application of BMCs improves recovery of left ventricular ejection fraction (LVEF) in patients with chronic heart failure.

Design, Setting, and Participants The CELLWAVE double-blind, randomized, placebo-controlled trial conducted among patients with chronic heart failure treated at Goethe University Frankfurt, Germany, between 2006 and 2011.

Interventions Single-blind low-dose (n=42), high-dose (n=40), or placebo (n=21) shock wave pretreatment targeted to the left ventricular anterior wall. Twenty-four hours later, patients receiving shock wave pretreatment were randomized to receive double-blind intracoronary infusion of BMCs or placebo, and patients receiving placebo shock wave received intracoronary infusion of BMCs.

Main Outcomes and Measures Primary end point was change in LVEF from baseline to 4 months in the pooled groups shock wave + placebo infusion vs shock wave + BMCs; secondary end points included regional left ventricular function assessed by magnetic resonance imaging and clinical events.

Results The primary end point was significantly improved in the shock wave + BMCs group (absolute change in LVEF, 3.2% [95% CI, 2.0% to 4.4%]), compared with the shock wave + placebo infusion group (1.0% [95% CI, −0.3% to 2.2%]) (P=.02). Regional wall thickening improved significantly in the shock wave + BMCs group (3.6% [95% CI, 2.0% to 5.2%]) but not in the shock wave + placebo infusion group (0.5% [95% CI, −1.2% to 2.1%]) (P=.01). Overall occurrence of major adverse cardiac events was significantly less frequent in the shock wave + BMCs group (n=32 events) compared with the placebo shock wave + BMCs (n=18) and shock wave + placebo infusion (n=61) groups (hazard ratio, 0.58 [95% CI, 0.40-0.85]; P=.02).

Conclusions and Relevance Among patients with postinfarction chronic heart failure, shock wave–facilitated intracoronary administration of BMCs vs shock wave treatment alone resulted in a significant, albeit modest, improvement in LVEF at 4 months. Determining whether the increase in contractile function will translate into improved clinical outcomes requires confirmation in larger clinical end point trials.

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REGENERATIVE THERAPIES HAVE emerged as a promising novel approach to improve heart function and prevent the development of end-stage heart failure.[1] Application of various cell types including bone marrow–, heart tissue–, or adipose tissue–derived cell populations were shown to improve cardiac functional recovery.[2-4] In patients with acute myocardial infarction, recent meta-analyses suggested a moderate but sustained enhancement of left ventricular function and improved clinical outcome following administration of bone marrow–derived mononuclear cells (BMCs).[5] In contrast, in patients with chronic postinfarction heart failure, BMC therapy has demonstrated heterogeneous results so far.[6-8] One possible reason for the impaired efficacy of cell therapy in the chronic setting is that cell retention in the heart is substantially reduced in comparison to acute myocardial infarction.[9] BMCs are attracted to the target tissue by cytokines such as

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stromal cell–derived factor 1 (SDF-1) and vascular endothelial growth factor.[10] The chemokine SDF-1 is only transiently expressed following acute myocardial infarction,[11] and in chronic ischemic models, expression of these chemoattractants is profoundly reduced, resulting in insufficient cell recruitment to the target area.[12] We have recently shown that extracorporeal application of focused low-energy shock waves increases the tissue expression of chemoattractants such as SDF-1 and vascular endothelial growth factor in the target tissues.[13] Our preclinical studies demonstrated that shock wave–induced local up-regulation of these chemoattractants resulted in significantly enhanced homing of applied cells, which translated into improved neovascularization of chronically ischemic tissue.

Thus, we hypothesized that shock wave–facilitated cell therapy improves the efficacy of intracoronary application of autologous BMCs in patients with chronic postinfarction heart failure.

METHODS

The study was a randomized, placebo-controlled, clinical phase 1/2 trial approved by the ethics committee and the Paul-Ehrlich-Institut, Langen, Germany (ID 183/01) and registered with clinicaltrials.gov. All patients provided written informed consent.

Patients were eligible for inclusion if they were aged 18 to 80 years with an anterior myocardial infarction occurring 3 months or more prior to inclusion and stable chronic postinfarction heart failure, defined as left ventricular ejection fraction (LVEF) less than 50% or symptoms of New York Heart Association (NYHA) class II or greater. Patients were also required to have a patent vessel supplying the target region. Major exclusion criteria were the presence of a ventricular thrombus and a baseline serum creatinine level greater than 2 mg/dL (176.8 μmol/L) in addition to poor ultrasound access to the heart. Detailed inclusion and exclusion criteria are listed in the eMethods at www.jama.com.

Randomization was performed in 2 steps for the entire study cohort at the cell processing center (German Red Cross Blood Service, Frankfurt) by a simple random allocation with known N (N = 100) using a computer list. Because of dropout after the first randomization step, 3 patients were added to the randomization list. Included patients were first randomized (2:2:1; single-blind) to receive echocardiographically guided low-dose, high-dose, or placebo shock wave targeted to the left ventricular anterior wall 24 hours prior to cell administration. Patients receiving shock wave pretreatment were then randomized in a second step (1:1; double-blind) to receive intracoronary infusion of either BMCs or cell-free medium (placebo), whereas patients receiving placebo shock wave received intracoronary infusion of BMCs (FIGURE 1). There was no blockwise randomization.

The primary efficacy end point was improvement of global LVEF on quantitative left ventricular angiography[7,14] at 4 months' follow-up. The absolute change in LVEF was compared between the pooled groups receiving shock wave + placebo infusion vs shock wave + BMCs.

Secondary end points included changes in global left ventricular volumes and in NYHA class as well as regional left ventricular function and late enhancement volume assessed by magnetic resonance imaging (MRI).[15] The clinical events death and mode of death, rehospitalization for worsening heart failure, recurrent myocardial infarction, ventricular tachycardia, revascularization, and stroke (major adverse cardiac events [MACEs]) were prospectively collected by study nurses.

Safety end points comprised the tolerance of shock wave application, occurrence of ventricular arrhythmias, and increases in troponin T levels after shock wave application, as well as in-hospital MACEs.

Shock Wave Application

Patients randomized to receive shock wave treatment received single-blind low-energy shock wave under continuous electrocardiographic trigger at either high dose (0.051 mJ/mm^2) or low dose (0.014 mJ/mm^2) to 4 separate spots in the target region (750 shots per spot; total, 3000 shots) under 2-dimensional echocardiographic guidance by a custom-built shock wave generator (Biotripter, Dornier Med Tech Systems). The shock waves were focused to the anterior, lateral, and apical left ventricular segments demonstrating severe wall motion impairment attributable to the previous myocardial infarction using modified parasternal long-axis and apical 4-chamber echocardiographic views. eFigure 1 illustrates the setup and principles of shock wave delivery. Placebo shock wave treatment was administered by placing an airfoam cushion between the shock wave applicator and the patient's chest wall, thus preventing shock wave penetration.

Cell and Placebo Preparation and Administration

Preparation of BMC and placebo infusions, and administration protocols were identical to those of the REPAIR-AMI trial.[4] Details are reported in the eMethods.

Statistics

Distributions of categorical variables were tested by χ^2 test or Fisher exact test. Continuous variables are reported as means and 95% CIs, if not stated otherwise. All variables were tested for homogeneity of variance by Levènes test. Groups were compared using analysis of variance. Paired variables were analyzed using *t* test. The primary statistical plan called for comparing absolute change in LVEF at 4 months between the shock wave + BMCs and shock wave + placebo infusion groups with a paired *t* test and $\alpha = 5\%$. The absolute improvement of 3% on the left ventricular function measurement scale at 4 months with an SD of 3 was the assumed effect size for our sample size calculation. Power analysis was conducted in STATA 11.2. A total of 20 patients ensured a statistical power of 80% based on our assumption, when comparing the treatment groups.

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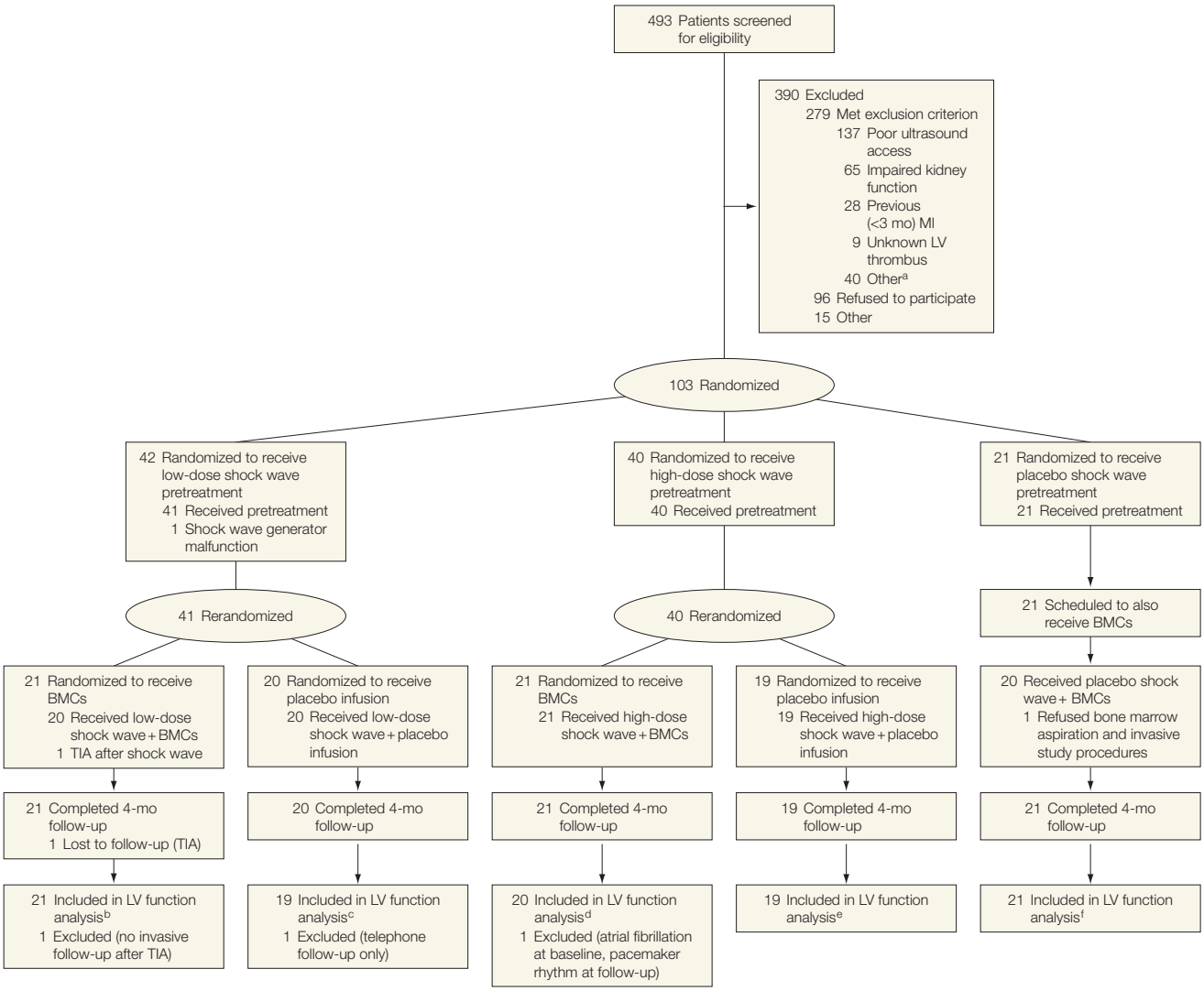
Preliminary data analyses suggested that patients with missing left ventricular angiography assessments tended to have a more severe disease course. We did not find any indication that the likelihood of missing data depends on unknown or unobserved variables. The exclusion of patients with no data on left ventricular angiography may result in biased effect estimates by a possible selection bias. We applied multivariate multiple imputation by an iterative Markov chain Monte Carlo approach[16] for estimating the missing data in left ventricular angiography. Predictor variables for missing values in LVEF, end-diastolic volume index, end-systolic volume index, and stroke volume index were any available left ventricular angiography assessment, age, sex, NYHA class, levels of creatinine and the N-terminal fragment of the precursor to B-type natriuretic peptide (NT-proBNP), and the Seattle Heart Failure Model score.

The results from the 5 imputed data sets were combined by the Rubin

Figure 1. Trial Design of CELLWAVE



BMCs indicates bone marrow–derived mononuclear cells; LV, left ventricular; MI, myocardial infarction; TIA, transient ischemic attack.
[a] See eMethods for reasons.
[b] Multiple imputations were applied for missing LV functional data in 3 cases: lost to follow-up at 4 months, new LV thrombus, and refusal of catheter.
[c] Multiple imputations were applied for missing LV functional data in 3 cases: non–ST-segment MI on follow-up, poor-quality LV angiography, and psychiatric disease.
[d] Multiple imputations were applied for missing LV functional data in 1 case: atrial fibrillation at baseline with pacemaker rhythm at follow-up.
[e] Multiple imputations were applied for missing LV functional data in 3 cases: 2 with poor-quality LV angiography and 1 with atrial tachycardia at baseline and sinus rhythm at follow-up.
[f] Multiple imputations were applied for missing LV functional data in 3 cases: 2 with poor-quality LV angiography and 1 refusal of invasive study procedures.

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method[17] to obtain the overall estimates, their standard errors, and 95% CIs. A total of 100 patients were included in the multiple-imputation approach. Left ventricular angiography was not performed in 3 patients at the 2 occasions because of transient ischemic attack after shock wave treatment, refusal of left ventricular angiography, and poor quality of the angiography on both occasions. Sensitivity analyses included the intention-to-treat approach. Prospective analyses included time to multiple and recurrent clinical events. Because more than 1 event may occur in the same patient, resulting in correlated failure times, the clinical events were ordered. The hazard ratios for ordered individual and combined clinical MACEs were estimated with the Anderson and Gill model[18] for recurrent time-to-event data.

The response pattern of change in LVEF between baseline and 4-month follow-up was investigated by a 2-step approach. A random-effects model[19] was applied for modeling the change in LVEF over time. An individual change index was predicted for each patient after fitting the random-effects model and categorized into the groups no change, improvement, and worsening. The resulting categorical variable was used as an outcome in multinomial logistic regression analyses testing for differences between the 5 treatment groups. The more patients were classified to a specific category, the more homogeneous was the change in LVEF.

$P < .05$ was considered significant, and tests were performed 2-sided. Analyses including the Anderson and Gill and the random-effects models were conducted in Stata version 11.2. All other analyses were performed using IBM SPSS Statistics version 20.

RESULTS

Patient Characteristics

A total of 103 patients were randomized to 5 different treatment groups (Figure 1). Baseline characteristics of these patients with chronic stable postinfarction heart failure are shown in TABLE 1. Mean time from the anterior myocardial infarction was 6 years, and mean NYHA class was II. Ten percent of patients received cardiac resynchronization therapy. All patients were taking maximal heart failure medication for at least 3 months prior to inclusion.

Safety of Shock Wave Application

Two-dimensional echocardiographically guided application of cardiac shock wave pretreatment was well tolerated. Continuous 24-hour telemonitoring of all patients following shock wave or placebo shock wave treatment revealed 1 case of nonsustained ventricular tachycardia, in a patient who had received low-dose shock wave treatment. Pacemaker and implantable cardioverter-defibrillator records did not show any device abnormalities or changes in programming after shock wave application. Serial measurement of troponin T levels detected no substantial increase in all but 1 patient, who sustained a non–ST-segment elevation myocardial infarction 18 hours after shock wave application and was treated successfully for a ruptured plaque in the mid–left anterior descending artery prior to intracoronary application of the study product. Other in-hospital events included 1 placement of an implantable cardioverter-defibrillator in the formerly mentioned patient with an episode of nonsustained ventricular tachycardia, 1 transient ischemic attack after low-dose shock wave application, and 1 surgical procedure of the groin to treat bleeding and hematoma formation after cardiac catheterization in a patient receiving low-dose shock wave therapy.

Effects on Left Ventricular Function by Quantitative Left Ventricular Angiography

Quantitative left ventricular angiography was performed in all but 2 patients at baseline. At 4 months' follow-up, left ventricular angiography data could not be obtained because of emergency intervention for non–ST-segment elevation myocardial infarction at follow-up (n=1), psychiatric disease (n=1), different heart rhythms at baseline and follow-up (n=2), unwillingness to repeat cardiac catheterization (n=3), and loss to follow-up (n=1). In addition, in 13 patients, the quality of the paired left ventricular angiography data was insufficient for analysis. In 9 of these patients, paired MRI data were available and used instead. Thus, in patients with missing left ventricular functional data at baseline or follow-up, values were estimated using multiple imputations. Thus, a total of 100 paired data sets (97%) for assessment of the primary and secondary end points were available (Figure 1).

As illustrated in TABLE 2, baseline LVEF did not significantly differ between the 5 groups. In the low-dose and high-dose shock wave + placebo infusion groups, there was no change in LVEF at 4 months. Likewise, there was also no significant change in LVEF in the placebo shock wave + BMCs group. In contrast, patients receiving intracoronary infusion of BMCs 24 hours after targeted low-dose or high-dose shock wave application demonstrated a significant improvement in LVEF at 4 months (Table 2 and FIGURE 2A).

For assessment of the primary end point, the high-dose and low-dose shock wave groups for patients receiving either BMCs or placebo infusion were combined. Analysis of the absolute change in LVEF from baseline to 4 months revealed that treatment with shock wave + BMCs was associated with a significant improvement in global LVEF (absolute change in LVEF, 3.2% [95% CI, 2.0% to 4.4%]; n=41) compared with shock wave + placebo infusion (absolute change in LVEF, 1.0% [95% CI, −0.3% to 2.2%]; n=38) (P=.02). Using only data obtained from quantitative angiography revealed identical results (absolute change in LVEF, 1.3% [95% CI, 0.03% to 2.5%]; n=31 for shock wave + placebo infusion vs 3.2% [95% CI, 2.1% to 4.3%]; n=33 for shock wave + BMCs [P=.02]).

As illustrated in Figure 2B, changes in LVEF in the prespecified subgroup of patients with a baseline LVEF of 40%

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or less revealed a dose-response effect ($P=.03$ for trend) from low-dose to high-dose shock wave pretreatment followed by BMC infusion. Moreover, LVEF improved in 27 of 29 patients receiving shock wave + BMCs, compared with only 18 of 28 patients in the shock wave + placebo infusion group ($P=.008$) and 9 of 14 patients in the placebo shock wave + BMCs group ($P=.02$).

In line with the results for LVEF, left ventricular stroke volumes demonstrated a significant increase in the high-dose shock wave + BMCs group but did not show a significant increase in the low-dose shock wave + BMCs group (Table 2). There were no significant changes in end-diastolic or end-systolic volumes at 4 months.

Regional Left Ventricular Function Assessed by MRI

Serial MRI analysis could be performed in 38 patients without implanted devices and free of claustrophobia. Overall analysis of left ventricular function confirmed the results of quantitative angiography for the primary end point (absolute change in LVEF from baseline to 4 months: −1.1% [95% CI, −5% to 3%]; n=12 for shock wave + placebo infusion; 1.9% [95% CI, 0% to 4%]; n=15 for shock wave + BMCs [$P=.13$]).

Wall thickening of infarcted segments improved significantly ($P=.01$ for trend) in patients receiving shock wave + BMCs (8.3% [95% CI, 5.7% to 10.9%] to 11.9% [95% CI, 8.9% to 14.9%]; $P<.001$) (FIGURE 3A) com-

Table 1. Baseline Characteristics

Characteristic	No. (%)					P Value
	Low-Dose Shock Wave + BMCs (n = 21)	Low-Dose Shock Wave + Placebo Infusion (n = 20)	High-Dose Shock Wave + BMCs (n = 21)	High-Dose Shock Wave + Placebo Infusion (n = 19)	Placebo Shock Wave + BMCs (n = 21)	
Men	17 (77)	16 (80)	18 (86)	17 (90)	16 (76)	.78
Age, mean (SD), y	65 (12)	60 (10)	58 (11)	63 (10)	60 (13)	.20
Hypertension	17 (77)	12 (60)	16 (76)	13 (68)	15 (71)	.75
Diabetes	11 (50)	2 (10)	9 (43)	7 (37)	6 (29)	.07
Smoking	14 (64)	12 (60)	17 (81)	13 (68)	16 (76)	.56
Hypercholesterolemia	17 (77)	17 (85)	17 (81)	16 (84)	17 (81)	.97
Vessels with disease, No.						
1	9	5	9	7	4	.43
2	7	3	5	5	6	
3	6	12	7	7	11	
Time from last myocardial infarction, median (IQR), mo	31 (8-88)	69 (15-142)	11 (4-126)	60 (16-185)	38 (6-83)	.22
Previous bypass surgery	4 (18)	7 (35)	6 (29)	5 (26)	8 (38)	.64
NYHA functional class						
I	1 (5)	4 (20)	2 (9)	2 (10)	2 (10)	.87
II	13 (59)	11 (55)	10 (48)	11 (58)	12 (57)	
III	8 (36)	5 (25)	9 (43)	6 (32)	7 (33)	
NT-proBNP, median (IQR), pg/mL	786 (345-1355)	1098 (616-2583)	1218 (367-2676)	878 (291-1760)	657 (336-982)	.22
Systolic blood pressure, mean (SD), mm Hg	118 (20)	110 (19)	113 (19)	108 (20)	114 (14)	.51
Heart rate, mean (SD), beats/min	67 (12)	66 (9)	66 (13)	68 (8)	69 (14)	.80
Left ventricular end-diastolic pressure, mean (SD), mm Hg	13 (6)	15 (7)	18 (9)	16 (8)	18 (8)	.20
Antiplatelet therapy	22 (100)	19 (95)	21 (100)	19 (100)	21 (100)	.38
Angiotensin-converting enzyme inhibitor/angiotensin II receptor blocker	20 (91)	20 (100)	21 (100)	16 (84)	21 (100)	.06
β-Blocker	18 (82)	20 (100)	19 (91)	19 (100)	21 (100)	.04
Statin	21 (96)	20 (100)	20 (95)	17 (90)	20 (95)	.67
Aldosterone antagonist	12 (55)	15 (75)	15 (71)	12 (63)	15 (71)	.62
Diuretics	17 (77)	19 (95)	19 (91)	16 (84)	16 (76)	.38
Digitalis	6 (27)	8 (40)	7 (33)	7 (37)	8 (38)	.92
Pacemaker/ICD	8 (36)	12 (60)	6 (29)	9 (47)	10 (48)	.31
Cardiac resynchronization therapy	2 (9)	2 (10)	1 (5)	2 (11)	3 (14)	.75
Applied number of BMCs, mean (SD), $\times 10^6$	150 (77)	NA	123 (69)	NA	90 (39)	.14
Applied number of cells giving rise to colonies, mean (SD), $\times 10^3$	32.6 (26.3)	NA	24.8 (23.3)	NA	22.1 (18.9)	.32
Therapy target vessel, No.						
Native coronary artery	18	16	18	16	16	.91
Bypass	3	4	3	3	5	

Abbreviations: BMC, bone marrow–derived mononuclear cells; ICD, implantable cardioverter-defibrillator; IQR, interquartile range; NA, not applicable; NT-proBNP, N-terminal pro-brain natriuretic peptide; NYHA, New York Heart Association.

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Table 2. Results From Quantitative Left Ventricular Angiography

	Mean (95% CI)					
	Low-Dose Shock Wave + BMCs (n = 21)	Low-Dose Shock Wave + Placebo Infusion (n = 19)	High-Dose Shock Wave + BMCs (n = 20)	High-Dose Shock Wave + Placebo Infusion (n = 19)	Placebo Shock Wave + BMCs (n = 21)	*P* Value From ANOVA
LVEF, %						
Baseline	37.2 (31.7 to 42.7)	29.9 (24.0 to 35.7)	32.4 (26.9 to 37.9)	32.3 (26.5 to 38.1)	33.4 (28.0 to 38.9)	.47
4 mo	39.9 (34.1 to 45.7)	30.2 (24.1 to 36.3)	35.5 (29.7 to 41.3)	34.0 (28.0 to 40.1)	34.4 (28.6 to 40.2)	.24
Absolute change	2.9 (1.2 to 4.6)	0.5 (−1.3 to 2.3)	3.5 (1.9 to 5.2)	1.5 (−0.4 to 3.3)	0.8 (−0.9 to 2.6)	.06
P value, baseline vs 4 mo	.001	.59	<.001	.12	.34	
End-diastolic volume index, mL/m²						
Baseline	93 (75 to 111)	123 (104 to 141)	105 (86 to 123)	111 (92 to 130)	92 (73 to 110)	.11
4 mo	96 (80 to 112)	118 (101 to 134)	102 (86 to 117)	114 (98 to 131)	94 (78 to 110)	.15
Absolute change	4 (−6 to 14)	−6 (−16 to 4)	−1 (−10 to 8)	6 (−4 to 15)	3 (−7 to 13)	.48
P value, baseline vs 4 mo	.40	.25	.78	.25	.56	
End-systolic volume index, mL/m²						
Baseline	59 (43 to 76)	91 (74 to 109)	73 (56 to 90)	79 (62 to 97)	63 (46 to 79)	.07
4 mo	59 (44 to 74)	86 (70 to 101)	67 (52 to 83)	79 (63 to 95)	63 (48 to 79)	.03
Absolute change	0 (−6 to 7)	−6 (−13 to 1)	−5 (−11 to 2)	2 (−5 to 9)	1 (−5 to 8)	.33
P value, baseline vs 4 mo	.92	.08	.16	.61	.70	
Stroke volume index, mL/m²						
Baseline	34 (29 to 38)	32 (26 to 37)	32 (27 to 36)	32 (27 to 37)	29 (24 to 34)	.77
4 mo	37 (31 to 42)	32 (27 to 37)	35 (30 to 40)	35 (30 to 41)	31 (26 to 36)	.45
Absolute change	4 (0 to 8)	1 (−4 to 5)	3 (0 to 7)	4 (0 to 8)	2 (−2 to 5)	.68
P value, baseline vs 4 mo	.06	.73	.04	.05	.44	

Abbreviations: ANOVA, analysis of variance; BMC, bone marrow–derived mononuclear cells; LVEF, left ventricular ejection fraction.

pared with those receiving shock wave + placebo infusion (8.5% [95% CI, 5.8% to 11.1%] to 8.9% [95% CI, 6.1% to 11.8%]; *P* = .55) and placebo shock wave + BMCs (5.1% [95% CI, 1.2% to 9.0%] to 7.0% 95% CI, 3.0% to 11.1%]; *P* = .02). Changes in regional wall thickening were paralleled by a significant (*P* = .001 for trend) decrease in global infarct size as measured by late enhancement volume (LEV) (5.1% [95% CI, 0.9% to 9.3%] for shock wave + placebo infusion; 1.1% [95% CI, −2.7% to 5.0%] for placebo shock wave + BMCs; −3.4% [−5.6% to −1.1%] for shock wave + BMCs) at 4 months. Even after exclusion of 1 patient experiencing a silent myocardial infarction in a area different than that treated in the left ventricle in the shock wave + placebo infusion group, there was a significant (*P* = .002 for trend) reduction in LEV in the shock wave + BMCs group compared with the shock wave + placebo infusion group (Figure 3B, eFigure 2).

Calculating global left ventricular scar mass from LEV and total left ventricular mass revealed that scar mass significantly decreased by an absolute −4.0 g (−6.6 to −1.3) in the shock wave + BMCs group but remained essentially unchanged in the shock wave + placebo infusion group (4.5 g [95% CI, −0.7 to 9.8]) and in the placebo shock wave + BMCs group (0.1 g [−4.4 to 4.6]) (*P* = .006 for trend). The alterations in scar mass were paralleled by reciprocal changes in left ventricular viable mass (−5 g [95% CI, −10 to 1] for shock wave + placebo infusion, −3 g [95% CI, −5 to 0] for placebo shock wave + BMCs, and 2 g [95% CI, −1 to 5] for shock wave + BMCs) (*P* = .007 for trend).

The absolute 3.4% reduction in LEV translated into a relative 10% smaller infarct size at 4 months in patients receiving shock wave + BMCs. By multivariable linear regression analysis (*P* < .001 from analysis of variance), treatment with shock wave + BMCs was a significant (*P* < .001) independent predictor of improved wall thickening of infarcted segments together with a smaller baseline segmental late enhancement (*P* < .001), whereas the transmurality of the scar (*P* = .09) or baseline wall thickening (*P* = .61) did not predict improvement in regional contractility.

Assessment of Heart Failure Status

Symptomatic heart failure status as assessed by NYHA classes remained unchanged from baseline to 4 months' follow-up in patients receiving low-dose or high-dose shock wave + placebo infusion (absolute change in NYHA class, 0.1 [95% CI, 0.4 to 0.5] in both groups), as well as in patients receiving placebo shock wave + BMCs (absolute change in NYHA class, −0.2 [95% CI, −0.6 to −0.1]). In contrast, patients receiving low-dose shock wave + BMCs showed a modest improvement in symptomatic status (absolute change in NYHA class, −0.3 [95% CI, −0.6 to 0]), and patients receiving high-dose shock wave + BMCs demonstrated a significant reduction in NYHA class (absolute change, −0.4 [95% CI, −0.8 to −0.1]) (FIGURE 4), driven by reductions in NYHA class III and corresponding increases in NYHA class I.

Baseline levels of NT-proBNP did not show statistically significant differ-

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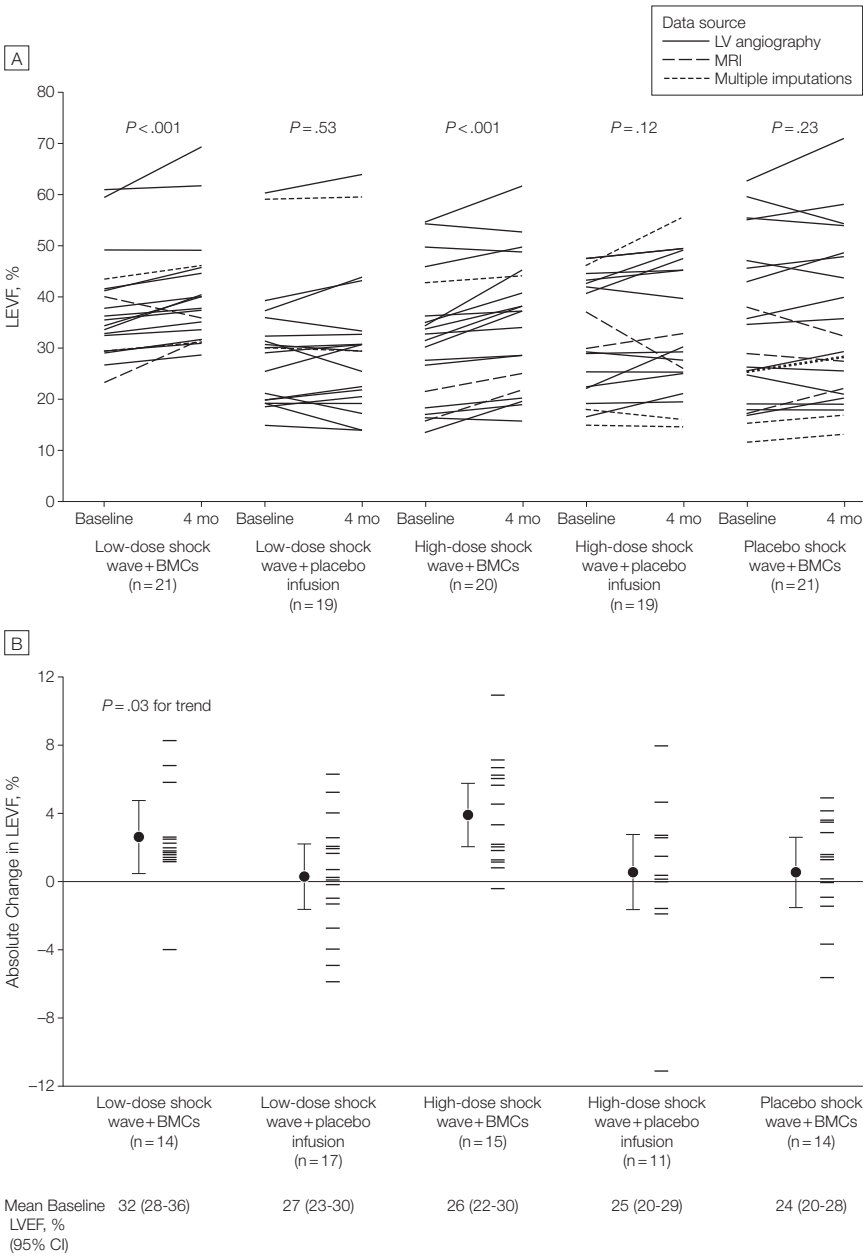
ences between the 3 pooled groups. Serial assessment of NT-proBNP serum levels was confined to patients with stable kidney function, defined as a creatinine level at 4 months lower than 125% of the baseline level. In the 29 patients receiving shock wave + placebo infusion, serum levels of NT-proBNP remained unchanged at 4 months' follow-up (1368 [95% CI, 780 to 1956] pg/mL at baseline vs 1174 [95% CI, 715 to 1633] pg/mL at 4 months) (P=.18). Likewise, there was no change in NT-proBNP level in the 18 patients receiving placebo shock wave + BMCs (669 [95% CI, 468 to 870] pg/mL at baseline vs 646 [95% CI, 433 to 858] pg/mL at 4 months) (P=.64). In contrast, in the 34 patients receiving shock wave + BMCs, NT-proBNP serum levels were significantly reduced from 1384 (95% CI, 847 to 1920) pg/mL at baseline to 1095 (95% CI, 662 to 1529) pg/mL at 4 months' follow-up (P=.04).

Figure 2. Left Ventricular Function Assessment: Left Ventricular Ejection Fraction



BMC indicates bone marrow–derived mononuclear cell; LV, left ventricular; LVEF, LV ejection fraction; MRI, magnetic resonance imaging. A, Absolute LVEF at baseline and at 4 months for each individual patient. P values obtained using paired t tests. B, Prespecified subgroup analysis of patients with baseline LVEF of 40% or less. Data markers indicate means; error bars, 95% CIs; horizontal lines, individual data. P for trend (from analysis of variance) is shown for the absolute change in LVEF.

Clinical Outcome

As shown in the analysis of multiple and recurrent clinical events (eFigure 3), the overall frequency of MACEs was significantly reduced in patients receiving placebo shock wave + BMCs (18 events) compared with patients receiving shock wave + placebo infusion (61 events) or shock wave + BMCs (32 events) (hazard ratio, 0.58 [95% CI, 0.40-0.85]; P=.02). FIGURE 5 illustrates the hazard ratios for the individual clinical end points and predefined combined end points comparing the shock wave + placebo infusion vs shock wave + BMCs groups. These data illustrate that the observed improvements in contractile left ventricular function and heart failure symptoms are paralleled by a decrease in the overall frequency of individual clinical end points.

DISCUSSION

The present clinical trial investigated the effects of combining target-tissue preconditioning by extracorporeal shock wave application with intracoronary infusion of autologous BMCs on left ventricular function in patients with chronic postinfarction heart-failure. The results demonstrate that shock wave–facilitated infusion of BMCs beneficially affects global and regional left ventricular contractile function and may reduce adverse clinical events in these chronically ill patients.

Preconditioning the target tissue by shock wave offers a novel approach to redirect intra-arterially applied cells to the region of interest by up-regulation of chemoattractant cytokines.[13] Local

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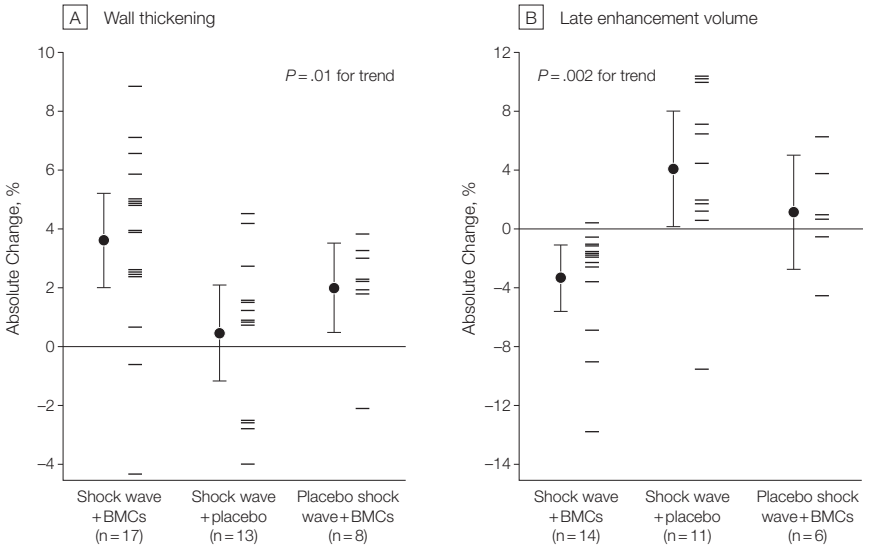
shock wave–induced up-regulation of SDF-1 is expected to improve homing and retention of C-X-C chemokine receptor type 4 (CXCR4)–expressing cells in the hearts of patients when the cells are delivered via the intracoronary route. Recently, Wu et al[20] demonstrated that early recruitment and retention of intramyocardially injected cardiac progenitor cells did predict subsequent contractile recovery in mouse models of myocardial infarction. Thus, attempts to increase recruitment and homing of applied cells should translate into improved efficacy. Indeed, the results of our study demonstrate that treatment with shock wave + BMCs was associated with a significant, albeit modest increase in LVEF attributable to improved wall thickening in the shock wave–treated region. Importantly, in the patients with the most severely reduced LVEF, treatment with shock wave + BMCs resulted in a homogeneous response, with improved LVEF observed in 93%, suggesting that target-region preconditioning reduces the heterogeneity in individual responses to intracoronary infusion of BMCs observed in the placebo shock wave + BMCs group of the present and in previous studies.[7] CXCR4, the SDF-1 receptor, is expressed in subpopulations of BMCs,[10,11,21] mesenchymal stem cells, very small embryonic-like cells, adult cardiac stem cells under hypoxic conditions, and in adipose tissue–derived stem cells.[22-24] Thus, preconditioning the target tissue by noninvasive application of shock waves may also improve the efficacy of other cell therapeutic approaches.

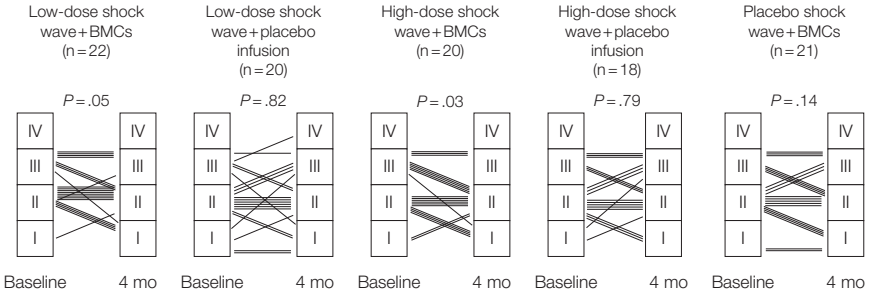
The 3.2% absolute improvement in global LVEF with shock wave–facilitated infusion of BMCs appears to be rather modest. However, as summarized in a recently published meta-analysis quantitatively assessing the relationship between short-term (4-6 months) therapy–induced changes in left ventricular remodeling analyzed in controlled trials and long-term outcomes in patients with heart failure attributable to left ventricular systolic dysfunction,[25] mean placebo-subtracted increases in LVEF range from 1.3% for valsartan in the Val-Heft (Valsartan Heart Failure Trial) including more than 5000 patients,[26] to 2.0% for aldosterone blockade,[27] 2.7% for cardiac resynchronization therapy,[25] and 2.9% for carvedilol in patients with stable heart failure and nonhibernating myocardium[28]—yet all of these therapies are well established to improve clinical outcomes in patients with chronic heart failure. These numbers compare favorably with the results of the present study showing a placebo-subtracted increase in LVEF of 2.0% for the entire study cohort and of 3.0% for the patients with LVEF less than 40%, which occurred in addition to comprehensive conventional pharmacological therapy for heart failure. Moreover, MRI revealed a selective increase in infarct wall thickening and a measurable reduction in infarct size in the shock wave + BMCs group. It is possible that, with time, these improvements could contribute to reversal of the adverse remodeling that commonly follows myocardial infarction. Nevertheless, the observed beneficial effects on MACEs during follow-up require confirmation in larger studies, because the

Figure 3. Left Ventricular Function Assessment: Wall Thickening and Late Enhancement Volume



Results obtained by magnetic resonance imaging. A, Absolute change in wall thickening within infarcted segments. B, Absolute change in late enhancement volume in percentage of left ventricular mass. Data markers indicate means; error bars, 95% CIs; horizontal lines, individual changes. *P* for trend is calculated using analysis of variance.

Figure 4. Results of NYHA Functional Class



Numbers in the boxes indicate New York Heart Association (NYHA) functional class, and each line indicates the change in NYHA class of an individual patient from baseline to 4 months' follow-up. *P* values were calculated using paired *t* test between NYHA classification at baseline and 4 months.

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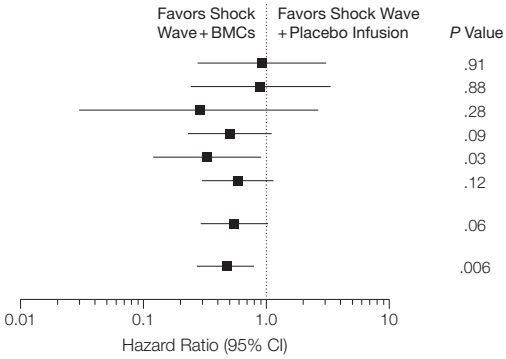
Figure 5. Hazard Ratios for Individual Clinical End Points and Predefined Combined End Points Comparing Shock Wave + Placebo Infusion vs Shock Wave + BMCs Groups

	No.				
	Shock Wave + BMCs (n = 43)	Shock Wave + Placebo Infusion (n = 39)	Hazard Ratio (95% CI)		*P* Value
All-cause death	5	6	0.9 (0.3-3.1)		.91
Cardiac death	4	5	0.9 (0.2-3.4)		.88
Repeat AMI	1	4	0.3 (0.03-2.7)		.28
Rehospitalization for CHF	9	20	0.5 (0.2-1.1)		.09
Ventricular tachycardia	5	16	0.3 (0.1-0.9)		.03
Cardiac death and rehospitalization for heart failure	13	25	0.6 (0.3-1.2)		.12
Cardiac death, rehospitalization for heart failure, and repeat AMI	14	29	0.6 (0.3-1.0)		.06
Cardiac death, rehospitalization for heart failure, repeat AMI and ventricular tachycardia	19	45	0.5 (0.3-0.8)		.006



Hazard ratios and 95% CIs for the individual and combined clinical major adverse cardiac events estimated by an Anderson and Gill model for multiple and ordered recurrent time-to-event data. BMCs indicates bone marrow–derived mononuclear cells.

sample size of the present study was not powered to demonstrate significant differences in clinical outcome.

Whereas treatment with shock wave + BMCs was associated with an increase in global and regional myocardial contractility, there was no significant improvement with shock wave + placebo infusion. Thus, in the setting of chronic postinfarction heart failure without ongoing ischemia, shock wave–mediated preconditioning alone does not appear to mediate contractile recovery by attracting endogenously circulating progenitor cells, which may be rationalized by the profound functional impairment of the cells in this patient cohort.[29]

Some limitations of our study merit further discussion. First and most importantly, the effects of BMC administration in the placebo shock wave group was considerably smaller than in the BMCs group of our previously published TOPCARE-CHD (Transplantation of Progenitor Cells and Regeneration Enhancement in Coronary Heart Disease) trial.[7] Side-by-side comparison of the 2 patient cohorts revealed that the patients in the present CELLWAVE trial had significantly more advanced heart failure and coronary artery disease compared with the TOPCARE-CHD patient cohort. In line with more advanced cardiac disease, the patients in the present study received significantly lower numbers of BMCs, and the administered cells had profoundly impaired colony-forming unit capacity, indicating severe functional impairment. Our previous studies demonstrated that the number of applied BMCs giving rise to colony-forming units is a major determinant of the efficacy of intracoronary infusion of BMCs in patients with chronic heart failure.[30] Thus, we believe that the more advanced cardiac disease has contributed to the lack of effect observed in the placebo shock wave + BMCs group of the present study.

Because the present study is to our knowledge the first to clinically apply focused shock wave therapy to the hearts of patients with postinfarction heart failure, it was designed to detect potential dose-dependent unwanted effects of shock wave application, such as minor myocardial injury or destabilization of coronary plaques, leading to acute coronary syndromes. Therefore, we purposely selected 2 doses of shock wave, corresponding to the medium and high doses of our experimental validation studies.[13] However, the prespecified analysis plan called for comparing the results of the combined low-dose and high-dose shock wave + BMCs vs shock wave + placebo infusion. The trial design also precluded a definite answer to the question of whether shock waves alone affected left ventricular contractile recovery, because we did not include a group of patients receiving both placebo shock wave and placebo infusion. Last, we used quantitative left ventricular angiography for the assessment of the primary end point of the study, although MRI would be superior. However, MRI is impossible in patients with implanted devices, so use of MRI would have precluded left ventricular analysis in almost half of our patient population. Nevertheless, MRI of suitable patients corroborated the findings obtained from left ventricular angiography and also provided important mechanistic insights.

In conclusion, shock wave–mediated preconditioning of the target tissue prior to intracoronary administration of autologous BMCs is associated with significant, albeit modest absolute improvements in global and regional left ventricular contractile function in patients with chronic postinfarction heart failure. However, the observed beneficial effects on clinical outcome require confirmation in larger clinical end point trials.

Author Contributions: Drs Assmus and Zeiher had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy

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of the data analysis. Drs Assmus and Walter contributed equally to the manuscript.
Study concept and design: Assmus, Walter, Lutz, Khaled, Dimmeler, Zeiher.
Acquisition of data: Assmus, Walter, Seeger, Leistner, Steiner, Ziegler, Khaled, Tonn, Zeiher.
Analysis and interpretation of data: Assmus, Walter, Klotsche, Tonn, Zeiher.
Drafting of the manuscript: Assmus, Walter, Lutz, Dimmeler, Zeiher.
Critical revision of the manuscript for important intellectual content: Assmus, Walter, Seeger, Leistner, Steiner, Ziegler, Khaled, Klotsche, Tonn, Dimmeler, Zeiher.
Statistical analysis: Assmus, Walter, Klotsche.
Obtained funding: Dimmeler, Zeiher.
Administrative, technical, or material support: Seeger, Steiner, Lutz, Khaled, Tonn, Dimmeler, Zeiher.
Study supervision: Assmus, Walter, Zeiher.

Conflict of Interest Disclosures: All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Dr Dimmeler and Dr Zeiher report that they are cofounders of t2cure, a for-profit company focused on regenerative therapies for cardiovascular disease, and for which they serve as scientific advisers and are shareholders. Dr Dimmeler reported serving on a scientific advisory board for Miragen; serving as a consultant to Merck; receiving grants or grants pending from the German Research Foundation (DFG) and the European Union; receiving payment for lectures from various entities; holding patents or patents pending (miR-29, miR-92); and receiving travel expenses from various entities. Dr Zeiher reported serving as a consultant to sanofi-aventis, Capricor, and Baxter Healthcare; receiving a grant from the DFG; receiving grants or grants pending from the German Ministry of Health and Education; receiving payment for lectures from Bayer, Berlin Chemie, Orbus Neich, and AstraZeneca; and holding patents or patents pending from Siemens Healthcare. No other authors reported disclosures.
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Online-Only Material: eMethods, eTable, and eFigures 1 and 2 are available at www.jama.com.
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REFERENCES

1. Steinhauser ML, Lee RT. Regeneration of the heart. *EMBO Mol Med*. 2011;3(12):701-712.
2. Bolli R, Chugh AR, D'Amario D, et al. Cardiac stem cells in patients with ischaemic cardiomyopathy (SCIPIO): initial results of a randomised phase 1 trial. *Lancet*. 2011;378(9806):1847-1857.
3. Makkar RR, Smith RR, Cheng K, et al. Intracoronary cardiosphere-derived cells for heart regeneration after myocardial infarction (CADUCEUS). *Lancet*. 2012;379(9819):895-904.
4. Schächinger V, Erbs S, Elsässer A, et al; REPAIR-AMI Investigators. Intracoronary bone marrow–derived progenitor cells in acute myocardial infarction. *N Engl J Med*. 2006;355(12):1210-1221.
5. Jeevanantham V, Butler M, Saad A, et al. Adult bone marrow cell therapy improves survival and induces long-term improvement in cardiac parameters: a systematic review and meta-analysis. *Circulation*. 2012; 126(5):551-568.
6. Kang HJ, Lee HY, Na SH, et al. Differential effect of intracoronary infusion of mobilized peripheral blood stem cells by granulocyte colony-stimulating factor on left ventricular function and remodeling in patients with acute myocardial infarction versus old myocardial infarction: the MAGIC Cell-3-DES randomized, controlled trial. *Circulation*. 2006;114(1)(suppl):I145-I151.
7. Assmus B, Honold J, Schächinger V, et al. Transcoronary transplantation of progenitor cells after myocardial infarction. *N Engl J Med*. 2006;355(12): 1222-1232.
8. Perin EC, Willerson JT, Pepine CJ, et al. Effect of transendocardial delivery of autologous bone marrow mononuclear cells on functional capacity, left ventricular function, and perfusion in chronic heart failure: the FOCUS-CCTRN trial. *JAMA*. 2012;307 (16):1717-1726.
9. Schächinger V, Aicher A, Döbert N, et al. Pilot trial on determinants of progenitor cell recruitment to the infarcted human myocardium. *Circulation*. 2008; 118(14):1425-1432.
10. Ceradini DJ, Kulkarni AR, Callaghan MJ, et al. Progenitor cell trafficking is regulated by hypoxic gradients through HIF-1 induction of SDF-1. *Nat Med*. 2004; 10(8):858-864.
11. Askari AT, Unzek S, Popovic ZB, et al. Effect of stromal-cell–derived factor 1 on stem-cell homing and tissue regeneration in ischaemic cardiomyopathy. *Lancet*. 2003;362(9385):697-703.
12. Penn MS. Importance of the SDF-1:CXCR4 axis in myocardial repair. *Circ Res*. 2009;104(10):1133-1135.
13. Aicher A, Heeschen C, Sasaki K, et al. Low-energy shock wave for enhancing recruitment of endothelial progenitor cells: a new modality to increase efficacy of cell therapy in chronic hind limb ischemia. *Circulation*. 2006;114(25):2823-2830.
14. Assmus B, Schächinger V, Teupe C, et al. Transplantation of Progenitor Cells and Regeneration Enhancement in Acute Myocardial Infarction (TOPCARE-AMI). *Circulation*. 2002;106(24):3009-3017.
15. Leistner DM, Fischer-Rasokat U, Honold J, et al. Transplantation of Progenitor Cells and Regeneration Enhancement in Acute Myocardial Infarction (TOPCARE-AMI): final 5-year results suggest long-term safety and efficacy. *Clin Res Cardiol*. 2011; 100(10):925-934.
16. Li K-H. Imputation using Markov chains. *J Stat Comput Simul*. 1988;30:57-79.
17. Rubin DB. *Multiple Imputation for Nonresponse in Surveys.* New York, NY: Wiley; 1987.
18. Andersen PK, Gill RD. Cox's regression model for counting processes. *Ann Stat*. 1982;10(4):1100-1120.
19. Laird NM, Ware JH. Random-effects models for longitudinal data. *Biometrics*. 1982;38(4):963-974.
20. Liu J, Narsinh KH, Lan F, et al. Early stem cell engraftment predicts late cardiac functional recovery: preclinical insights from molecular imaging. *Circ Cardiovasc Imaging*. 2012;5(4):481-490.
21. Seeger FH, Rasper T, Koyanagi M, et al. CXCR4 expression determines functional activity of bone marrow–derived mononuclear cells for therapeutic neovascularization in acute ischemia. *Arterioscler Thromb Vasc Biol*. 2009;29(11):1802-1809.
22. Sovalat H, Scrofani M, Eidenschenk A, et al. Identification and isolation from either adult human bone marrow or G-CSF-mobilized peripheral blood of CD34(+)/CD133(+)/CXCR4(+)/Lin(−)CD45(−) cells, featuring morphological, molecular, and phenotypic characteristics of very small embryonic-like (VSEL) stem cells. *Exp Hematol*. 2011;39(4):495-505.
23. Tang YL, Zhu W, Cheng M, et al. Hypoxic preconditioning enhances the benefit of cardiac progenitor cell therapy for treatment of myocardial infarction by inducing CXCR4 expression. *Circ Res*. 2009; 104(10):1209-1216.
24. Baek SJ, Kang SK, Ra JC. In vitro migration capacity of human adipose tissue–derived mesenchymal stem cells reflects their expression of receptors for chemokines and growth factors. *Exp Mol Med*. 2011; 43(10):596-603.
25. Kramer DG, Trikalinos TA, Kent DM, et al. Quantitative evaluation of drug or device effects on ventricular remodeling as predictors of therapeutic effects on mortality in patients with heart failure and reduced ejection fraction. *J Am Coll Cardiol*. 2010; 56(5):392-406.
26. Wong M, Staszewsky L, Latini R, et al. Severity of left ventricular remodeling defines outcomes and response to therapy in heart failure: Valsartan Heart Failure Trial (Val-HeFT) echocardiographic data. *J Am Coll Cardiol*. 2004;43(11):2022-2027.
27. Ezekowitz JA, McAlister FA. Aldosterone blockade and left ventricular dysfunction: a systematic review of randomized clinical trials. *Eur Heart J*. 2009; 30(4):469-477.
28. Cleland JG, Pennell DJ, Ray SG, et al; Carvedilol Hibernating Reversible Ischaemia Trial: Marker of Success Investigators. Myocardial viability as a determinant of the ejection fraction response to carvedilol in patients with heart failure (CHRISTMAS trial). *Lancet*. 2003;362(9377):14-21.
29. Kissel CK, Lehmann R, Assmus B, et al. Selective functional exhaustion of hematopoietic progenitor cells in the bone marrow of patients with postinfarction heart failure. *J Am Coll Cardiol*. 2007;49(24):2341-2349.
30. Assmus B, Fischer-Rasokat U, Honold J, et al; TOPCARE-CHD Registry. Transcoronary transplantation of functionally competent BMCs is associated with a decrease in natriuretic peptide serum levels and improved survival of patients with chronic postinfarction heart failure. *Circ Res*. 2007;100(8):1234-1241.

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